with a bi-molecular probing mode of action, whereas substantial allele specific signals were produced in the Scorpions reactions. It is worth noting that the final level of signal for the heterozygote was half that generated by the homozygous C amplification. This experiment illustrates the substantial kinetic advantages of the unimolecular hybridisation approach of this invention.

**Examples 7 and 8**

**Random coil embodiment and bimolecular embodiment**

Scorpion B2731:

fam-AGGTAGTGCAGAGAGTG-mr-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC

Scorpion B4249 (no quencher on same molecule)

fam-AGGTAGTGCAGAGAGTG-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC

Quencher oligonucleotide (complement of the tail of B4249):

CACTCTCTGCACTACCT-mr

ARMS primer R284-97: TTCGGGGCTCCACACGGCGACTCTCAAC

ARMS primer R283-97: TTCGGGGCTCCACACGGCGACTCTCAAG

Target is the H63D polymorphism of the human heriditary haemochromatosis gene (HH), B2731 and B4249 are "common" primers to oppose the ARMS primers R283-97, R283-97. Cycling conditions and reaction composition as above. Primers (including *Scorpion* primers) were used at 500nM concentration.

For the two molecule example, the quencher oligonucleotide was incorporated at 0, 0.5 2 and 20 mM, that is: 0, 1, 4, 40 fold relative to the Scorpion primer.

The random coil embodiment (Figure 19) confirms that random coiling alone can be sufficient to bring the probe and quencher together and that an increase in signal is readily obtained in continuous monitoring of PCR. (Furthermore, it should be noted that this particular amplicon had previously proven refractory to probing in a TaqMan or Molecular Beacons assay).

The bimolecular embodiment also gave good results (see Figures 20 and 21). The more quencher was added, the lower the backgrounds in the absence of amplicon. The

optimal overall performance (taking account of absolute signal strength and signal/noise) was with equimolar and 4x excess quencher.

**Example 9**

**No quencher embodiment**

Scorpion B4249 (no quencher)

fam-AGGTAGTGCAGAGAGTG-h GAGCCTCAACATCCTGCTCCCCTCCTACTAC

ARMS primer R284-97

TTCGGGGCTCCACACGGCGACTCTCAAC

Reactions were set up as described in the previous examples. Primers were included at 500nM. The target was the H63D mutation of the human hereditary haemochromatosis gene (HH). 25ng of DNA was added per reaction. B4249 was the common primer in combination with the ARMS mutant primer R284-97. Cycling was as described in the previous examples. The results are shown in Figure 18.

In this example, mutation specific signal was generated in the absence of a quencher. Random folding of the *Scorpion* primer around the fluorophore provides sufficient quenching of the fluorophore. An increase in signal is readily obtained during continuous monitoring of PCR.